

1. A non-functional BADH2 form was detected in *P. amaryllifolius*. In scented rice and other 2AP synthesizing systems, such non-functional form of BADH2 is reported that blocks the GABA synthesis and leads in the accumulation of  $\Delta^1$ pyrroline, an immediate precursor of 2AP. The present finding of non-functional form of BADH2 suggests similar mechanism of biosynthesis of 2AP in *P. amaryllifolius*. This is the first report of its kind in *P. amaryllifolius*.
2. In addition to non-functional BADH2 form, functional BADH2 was also detected in *P. amaryllifolius*. Thus the study showed the co-existence of functional and nonfunctional BADH2 gene isoforms in *P. amaryllifolius*. This is also a first report of its kind in *P. amaryllifolius*.
3. The higher relative expression of BADH2 along with 2AP synthesis supports the co-existence of two forms of BADH2 gene in *P. amaryllifolius*.
4. The higher *in vivo* BADH2 enzyme activity also supports the existence of functional BADH2 gene in *P. amaryllifolius*.
5. The *in vivo* enzyme assay confirms  $\gamma$ -amino butyraldehyde as the preferential substrate for BADH2 in *P. amaryllifolius*.
6. The recombinant PaBADH2 protein activity also confirmed the substrate specificity with  $\gamma$ -amino butyraldehyde in *P. amaryllifolius*.
7. The 9-10 pH optima of PaBADH2 suggests that the protein might be targeted in peroxisomes.
8. The docking studies of PaBADH2 confirmed that it belongs to the ALDH\_SF superfamily with a specific hit with the ALDH\_F10\_BADH, i.e. betaine aldehyde dehydrogenase family.
9. The docking studies also indicated that the key regions for the substrate binding pocket, cofactor binding domain and oligomerization domain are conserved in *P. amaryllifolius*.
10. The Ramchandran plot analysis showed that the PaBADH2 protein model had good stereochemical quality with 91.3 % of amino acid residues present in most favored regions.
11. The partial and hybrid structures of PaBADH2 showed the same interaction energies with the substrates, suggesting that the partial PaBADH2 is active in *P. amaryllifolius*.

12. The histochemical studies explained the site specific expression of two forms of BADH2. The functional form is active in the mesophyll tissue while the non-functional form is present in the epidermal tissue where 2AP is stored.
13. The highest 2AP synthesis in *P. amaryllifolius* can be correlated with higher contents of its two precursors - proline and methylglyoxal than in scented rice.
14. Thus, overall the present study explains the molecular mechanism of 2AP expression in *P. amaryllifolius* that seems to be similar as found in scented rice.